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# ID2 mediates the transforming growth factor-β1-induced Warburg-like effect seen in the peritoneum of women with endometriosis

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**Running title:**

ID2 and TGF-β1 regulate metabolism in endometriosis

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## Abstract

**Study question**: Is inhibitor of DNA binding protein 2 (ID2) a mediator of the transforming growth factor (TGF)- $\beta$ 1-induced Warburg-like effect seen in the peritoneum of women with endometriosis?

**Study finding:** The TGF-β1-induced changes in the metabolic phenotype of peritoneal mesothelial cells from women with endometriosis are mediated through the ID2 pathway.

**What is known already:** TGF-β1 induces the metabolic conversion of glucose to lactate via aerobic glycolysis (the 'Warburg effect') in the peritoneum of women with endometriosis, through increased expression of the transcription factor hypoxia inducible factor α (HIF-1α). ID proteins are transcriptional targets of TGF-β1.

**Study design, samples/materials, and methods:** Expression of ID2 was investigated in luteal phase peritoneal biopsies from women with regular menstrual cycles, with and without endometriosis (n=8-10 each group) by quantitative RT–PCR (qRT-PCR) and immunohistochemistry. *ID2* mRNA expression in primary human peritoneal mesothelial cells (HPMC) and immortalized mesothelial cells (MeT-5A) was assessed by qRT-PCR (n=6). The effects of TGF-β1 and ID2 siRNA on HIF-1α mRNA expression and lactate secretion was assessed using qRT-PCR and a colorimetric lactate assay**.**

**Main results and the role of chance:** ID2 is localised to peritoneal mesothelial and stromal cells of women with and without endometriosis. *ID2* mRNA expression is lower in peritoneum adjacent to the endometriosis lesions compared to distal sites ($p<0.01$). Exposure of HPMC and MeT-5A cells to physiological concentrations of TGF-β1 decreases *ID2* mRNA expression ($P<0.01$, $P<0.001$, respectively, versus

control). ID2 knockdown increases HIF-1α mRNA expression ($p<0.01$) and lactate secretion ($p<0.05$ versus scrambled control) to the same degree as with exposure to TGF-β1.

**Limitations, reasons for caution:** Primary human cell cultures and a cell line were used in this study, and thus the results may not fully represent the situation *in vivo*. The results should also be replicated using a larger number of samples**.**

**Wider implications of the findings:** Novel therapeutics that target the TGFβ/ID pathway offer a potential role in the treatment of endometriosis.

**Large scale data:** N/A

**Study funding and competing interest(s):** This work was funded by a Wellbeing of Women research grant (R42533) awarded to AWH, JKB and WCD; and an MRC Centre Grant G1002033. VJY received grant support from Federation of Women Graduates (134225) and a PhD studentship from the College of Medicine and Veterinary Medicine at the University of Edinburgh. There are no competing interests to declare.

**Key Words:**

endometriosis, lactate, hypoxia inducible factor-1α, inhibitor of DNA binding protein 2, mesothelium

## Introduction

Endometriosis is a benign inflammatory disorder, defined by the presence of endometrial tissue outside the uterus with lesions typically found on the pelvic peritoneum in close association with the peritoneal mesothelium (Giudice, 2010). The prevalence of endometriosis is estimated at 2-10% of women of reproductive age and it is associated with chronic pelvic pain, dysmenorrhoea, dyspareunia and infertility (Giudice, 2010). Surgical excision can provide symptom relief, but symptoms recur in up to 75% of surgical cases and available medical treatments have undesirable side effects and are contraceptive (Jacobson, et al., 2009). The aetiology of endometriosis is uncertain. However, increasing evidence suggests endometriosis lesions may share characteristics with tumours (including resistance to apoptosis, angiogenesis and invasion) and changes in the peritoneal microenvironment are thought to contribute to the pathophysiology of this disease (Jacobson, Duffy, Barlow, Koninckx and Garry, 2009, Young, et al., 2014, Young, et al., 2013).

Aberrant expression of transforming growth factor-β1 (TGF-β1) is well defined during tumorigenesis and has been shown to induce 'the Warburg effect', the metabolic conversion of glucose to lactate in normoxia (Fosslien, 2008). High levels of energy rich lactate 'feed' cancer cells allowing them to survive while increasing angiogenesis, local inflammation and resistance to apoptosis, further fuelling tumour progression and metastasis (Hirschhaeuser, et al., 2011). The Warburg effect is induced by inflammatory cytokines, including TGF-β1, through induction of the transcription factor hypoxia inducible factor α (HIF-1α) under normoxic conditions (Fosslien, 2008). We have previously shown that endometriosis lesions and the

surrounding peritoneum use aerobic glycolysis as a means of energy production in a similar fashion to the Warburg effect in tumorigenesis (Young, et al., 2014). Furthermore, we found TGF-β1 initiated Warburg-like metabolism, through induction of HIF-1α protein in aerobic conditions, in the peritoneal mesothelial cells. Increased lactate levels, a by-product of glycolysis, were observed within the peritoneal fluid of women with endometriosis (Young, et al., 2014). Overproduction of lactate increases cell invasion, angiogenesis and immune suppression, all crucial steps in the development of tumors and known regulators of endometriosis (Hirschhaeuser, Sattler and Mueller-Klieser, 2011). Importantly, peritoneal fluid lactate levels directly correlated with TGF-β1 concentrations (Young, et al., 2014).

TGF-β1 has been reported to regulate HIF-1α mRNA and protein expression via the inhibitor of DNA binding (ID) proteins in epithelial cells (Cao, et al., 2009). The IDs are basic helix-loop-helix (bHLH) proteins that lack a DNA-binding domain but can heterodimerise with other bHLH transcription factors, such as HIF-1α, preventing them from binding to DNA. This interaction can therefore have positive or negative effects on gene expression (Ruzinova and Benezra, 2003). In particular, ID2 is reported to be down-regulated by TGF-β signalling in epithelial cells and has been shown to be involved in the TGF-β induced epithelial to mesenchymal transition and increased tumour invasion (Kondo, et al., 2004). Much of this evidence is based on decreased ID2 protein increasing HIF-1α mRNA expression (Tsai and Wu, 2012) and overexpression of ID2 has been shown to block HIF-1α protein activation (Cao, et al., 2009).

Here, we investigate the regulation and effects of ID2 expression in peritoneal mesothelial cells. In particular, we investigate whether TGF-β1 regulated ID2 may account for increases in HIF-1α expression and changes to peritoneal mesothelial cell metabolism in women with endometriosis.

## Methods

### Patients

Ethical approval for this study was obtained from the Scotland A Research Ethics Committee (LREC 11/AL/0376). Informed written consent was obtained from all patients and all tissues were collected according to EPHect guidelines (Fassbender, et al., 2014) and as previously described (Young, et al., 2014). Tissue biopsies were collected from women with peritoneal (no evidence of ovarian or deep disease) endometriosis (who had macroscopic evidence of endometriosis at laparoscopy and where the diagnosis was confirmed by post-operative histological examination of the lesions) and women without endometriosis (who displayed no evidence of endometriosis or any other underlying pelvic pathology at laparoscopy). None of the women were taking hormonal contraceptives at the time of tissue collection and they all had regular 21-35 day menstrual cycles. All samples were collected in the luteal phase of the cycle. In the women with endometriosis, we collected peritoneal biopsies from peritoneum adjacent (1-3cm away from) (n=10) and distal (outside the pelvic brim) (n=10) to endometriosis lesions. In the women without endometriosis, we collected peritoneal biopsies from sites prone to endometriosis within the ovarian fossae (n=8). Histological examination confirmed the absence of endometriosis lesions in the peritoneal biopsies. Primary human peritoneal mesothelial cells (HPMC) were collected at the time of surgery from women with and without endometriosis as previously described (Young, et al., 2014) by gently brushing the pelvic mesothelium with a Tao$^{TM}$ brush (QC Sciences, VA, USA).

**Cell culture**

HPMC were cultured in HOSE1 media containing; 40% media 199, 40% MCDB 105 and supplemented with 15% fetal bovine serum (FBS), 0.5% penicillin/streptomycin and 1% L-glutamine, at 37°C under 5% $CO_2$ in air (Life Technologies Inc., Paisley UK and Sigma Chemical Co., Poole UK). The MeT-5A mesothelial cell line (CRL-9444, ATCC, Middlesex, UK) was cultured in Iscove's Modified Dulbecco's Media (IMDM) supplemented with 10% FBS and 1% L-glutamine at 37°C under 5% $CO_2$ in air (Life Technologies Inc.). HPMC and MeT-5A cells were plated at $1.5x10^5$ or $2x10^5$ cells/ml, respectively, in a 12 well plate and left to adhere for 12 hours before being serum starved for 24 hours. Cells were exposed to physiological levels of recombinant human TGF-β1 (2ng/ml) or, as mesothelial cells are a source of TGF-β1 (Young, et al., 2014), to a neutralising TGF-β antibody (0.5μg/ml) (R&D Systems, Abingdon, UK) for between 3hr and 48hr.

**siRNA knockdown**

For siRNA knockdown, MeT-5A cells were plated at 3 x $10^5$ cells/well in a six-well culture plate with ID2 siRNA (two different siRNA sequences were combined for optimal knockdown) (Table 1) or scrambled siRNA (SlincerR, Ambion) using the siPORT neofection transfection kit (ThermoFisher Scientific, Loughborough, UK) for 48 hours. Successful transfection conditions were developed using positive control GAPDH siRNA where reduced gene expression was confirmed at the mRNA level by quantitative RT-PCR (qRT-PCR), at the protein level by Western blotting and cytotoxicity was confirmed to be less than 15% using a lactate dehydrogenase assay

(Source Bioscience, Nottingham, UK) (data not shown). Successful ID2 knockdown was confirmed by western blot and by qRT-PCR.

**Immunohistochemistry**

Peritoneal biopsies (n=3 in each clinical group) were examined by immunohistochemistry, as previously described (Young, et al., 2014). Briefly, sections of paraffin embedded tissue were mounted onto microscope slides and dewaxed and rehydrated before antigen retrieval in 10mM Tris, 1mM EDTA pH 9 with 5 min of pressure-cooking. This was followed by incubation with 3% hydrogen peroxide for 30 min and blocking in normal horse serum diluted 1:12 in Tris buffered saline with 0.5% Tween 20 (TBST20) for 30min. Slides were incubated with primary antibody overnight at 4°C (ID2 Santa Cruz sc-489 diluted 1:500, isotype match control Rabbit IgG Dako X0903) and then washed in TBST20 before incubation with species specific impress kit for 30 min at room temperature (Vector Laboratories, Peterborough, UK). Slides were then washed and incubated with 3, 3'-diaminobenzidine for 5min and counterstained with hematoxylin, dehydrated and visualized by light microscopy, using an Olympus Provis microscope equipped with a Kodak DCS330 camera (Olympus Optical Co., London, UK, and Kodak Ltd., Herts, UK). Due to the limited supply of peritoneal tissue, both positive and negative controls were performed on endometrial tissue.

**Immunoblotting**

Cell lysates (at a concentration of 200,000 cells/200µl, with 10µl loaded onto each lane) were resolved on NuPAGe Novex 4-12% Bis-Tris polyacrylamide gels under reducing conditions with NuPAGE MOPS sodium dodecyl sulphate running buffer

and according to the manufacturers' instructions (Life Technologies Inc.). Proteins were transferred to a polyvinylidene difluoride membrane using a semi-dry blotter and blocked with 5% milk powder in Tris-buffered saline with 0.1% Tween 20 (TBST-20). The membrane was incubated with mouse anti-GAPDH (Sigma G9545 0.5µg/ml) in TBS-T20 and 5% milk for 2 hours at room temperature, before incubating with anti-ID2 (SantaCruz Sc-489, 1µg/ml) in TBS-T20 and 5% milk overnight at 4°C. Following this membranes were washed and incubated with species specific impress kit 1:10000 in TBS-T20 and 5% milk for 1 hour at room temperature (Vector Laboratories Ltd., Peterborough, UK). The membrane was washed and incubated with Tyramide for 30 minutes and imaged using a FujiFilm FLA-5100 Fluorescent Image Analyzer (PerkinElmer, Cambridgeshire, UK and Fujifilm Ltd., Bedford, UK).

**Transcript analysis**

RNA was isolated from all tissues/cells using the RNeasy Mini/maxi kit and cDNA synthesis was performed using Superscript VILO Master Mix, according to manufacturer's instructions (Qiagen, West Sussex, UK; Life Technologies). Quantitative RT-PCR reactions were performed using brilliant III ultra-fast SYBR green QPCR master mix with standard running conditions on an ABI Prism 7900 Fast system (Agilent, Berkshire, UK, Applied Biosystems, Warrington, UK)**.** Pre-validated primers were used and melt curves were analyzed to confirm specific products (Table 2) (Primardesign, Southampton, UK). Messenger RNA transcripts were quantified relative to the appropriate housekeeping gene *GAPDH* as determined by geNorm assay and using the $2^{-\Delta Ct}$ or the $2^{-\Delta\Delta Ct}$ method.

**Lactate assay**

Lactate concentration in MeT-5A conditioned media was determined as previously described (Young, et al., 2014) using a commercial kit adapted for use on a Cobas Fara centrifugal analyzer (Roche Diagnostics Ltd., Welwyn Garden City, UK). The linear range was 0.15 - 19 mmol/l, and the within and between batch coefficients of variation were 0.9% and 2.7%, respectively.

**Statistical analysis**

All the results are from a minimum of three independent experiments expressed as mean ± SEM. Quantitative RT-PCR and lactate assay were analysed using students' *t* tests or one-way ANOVA and Tukeys post-test to compare each group, generated using GraphPad PRISM version 5 (GraphPad Software Inc., La Jolla, CA, USA) statistical software and a $p$ value of $<0.05$ was considered significant.

## Results

### HPMC express ID2

ID2 protein was localised to the mesothelial cells of the peritoneum from women with and without endometriosis (Figure 1A). Endometrial tissue was used as positive control and no staining was observed in the isotype-matched negative control (data not shown). To investigate whether ID2 is differentially expressed in the peritoneum of women with endometriosis, *ID2* expression was quantified by RT-PCR in peritoneal biopsies from the sites adjacent and distal to endometriosis lesions from women with endometriosis. We found that *ID2* expression was decreased in the peritoneum adjacent to endometriosis lesions when compared to peritoneum distal to endometriosis lesions ($P<0.05$; Figure 1B). In addition, both primary cultures of HPMC and the peritoneal mesothelial cell line (MeT-5A) expressed *ID2* mRNA (Figure 1C, D).

### TGF-β1 down-regulates *ID2* mRNA in HPMC

In order to assess the effect of TGF-β1 on *ID2* expression in mesothelial cells, HPMCs and MeT-5a cells were exposed to physiological concentrations of TGF-β1 (2ng/ml). Exposure of HPMC to TGF-β1 for 12 hours decreased *ID2* mRNA expression ($P<0.01$ versus control; Figure 1D). Exposure of MeT-5A cells to TGF-β1 for 3, 6, 12 and 24 hours showed that TGF-β1 induced a rapid and sustained reduction in *ID2* mRNA ($P<0.01$-$P<0.001$ versus control; Figure 1E). Since mesothelial cells are a source of TGF-β1, for control purposes an inhibition of TGF-$\beta$ activity by the anti-TGF-$\beta$ antibody was confirmed by immunostaining for phosphorylated Smad 2/3. Immunostaining showed that treatment with anti-TGF-$\beta$ antibody, at 0.5ug/ml as

recommended by the manufacturer, resulted in no positive staining of phosphorylated Smad 2/3 within the cell nucleus (Supplementary Figure 1).

**ID2 knockdown mimics TGF-β actions**

ID2 knockdown was confirmed at protein and mRNA level (Figures 2A and 2B, respectively). *ID2* mRNA decreased after siRNA knockdown, in the presence or absence of TGF-β1 (Figure 2B). ID2 siRNA knockdown significantly increased HIF-1α mRNA expression in MeT-5A cells. (Figure 3A). ID2 siRNA knockdown also significantly increased lactate production (Figure 3B). TGF-β1 alone, or in combination with ID2 siRNA, appeared to cause an increase in HIF-1 $\alpha$ mRNA and lactate secretion (no statistical analysis). ID2 knockdown mimics the actions of TGF-β1 treatment as it upregulates HIF-1α expression and lactate secretion, both in HPMC and MeT-5a cells (Figure 3A, 3B).

## Discussion

In this study, we demonstrated that ID2 protein is expressed in peritoneal mesothelial cells from women with and without endometriosis and that *ID2* expression is decreased in HPMC and MeT-5A cells upon exposure to physiological concentrations of TGF-β1. We also found that there is a local decrease in *ID2* expression in the peritoneum adjacent to endometriosis lesions, consistent with locally increased TGF-β1 activity. Using siRNA, we demonstrate that decreasing levels of ID2 induce a significant increase in HIF-1α expression and lactate expression in MeT-5A cells, similar to that seen with TGF-β1.

We have previously shown that TGF-β1 induces a change in metabolism from oxidative phosphorylation to aerobic glycolysis in peritoneal mesothelial cells adjacent to endometriosis lesions (Young, et al., 2014), a phenomenon which is known as 'Warburg Effect' in tumorigenesis and has been shown to promote cell invasion, angiogenesis and immune suppression (Vander Heiden, et al., 2009). Interestingly, these are all crucial steps in the development and progression of endometriosis. Our results presented here suggest that TGF-β1 regulates changes in metabolism in the peritoneal mesothelial cell through an ID2 - HIF-1α pathway.

TGF-β1 is a known regulator of *ID* gene expression in a variety of cells including immune cells, endothelial cells and epithelial cells (Ruzinova and Benezra, 2003). In epithelial cells, TGF-β, signalling through the Smad 2/3 pathway, classically inhibits *ID2* by activating the transcriptional repressor ATF3 (activating transcription factor 3) which in turns binds to the ATF/CREB (cAMP response element-binding protein) site

within the *ID2* promoter suppressing transcription (Kang, et al., 2003). Overexpression of TGF-β1 in breast cancer leads to a significant decrease in ID2 and this is linked to an increase in cell proliferation, invasion and extracellular matrix (ECM) remodelling (Itahana, et al., 2003). Additionally, TGF-β1 has been shown to prevent apoptosis and induce proliferation in endometrial stromal cells (Rahimi and Leof, 2007), to increase adhesion of normal human endometrial stromal cells to mouse peritoneum (Beliard, et al., 2003) and to induce the invasion of ectopic endometrial cells by facilitating the matrix metalloproteinases (MMPs) in the peritoneal mesothelial cells via ECM remodelling in peritoneal adhesion formation (Ma, et al., 1999). In the present study, we have shown that knockdown of ID2 and addition of TGF-β1 induced lactate secretion from the mesothelial cells (i.e. a Warburg-like effect). Of importance is that increased lactate secretion has been shown to increase proliferation, adhesion and invasion in tumor cells and these are also the key steps in the development and progression of endometriosis. Thus, increased concentrations of TGF-β1 in the peritoneal fluid and peritoneum of women with endometriosis (Young, et. al., 2014) may decrease the expression of *ID2* in the peritoneal mesothelial cells of women with endometriosis in a similar fashion. Decreased ID2 may increase bHLH transcription factors action and thus contribute to lesion development by increasing the invasiveness of the peritoneal mesothelial cells and inducing peritoneal ECM remodelling (Young, et al., 2013).

We also demonstrate that knockdown of ID2 increases lactate secretion in peritoneal mesothelial cells, suggesting that ID2 may have effects on both HIF-1α mRNA expression and protein activation. This observation is supported by findings in epithelial cells where overexpression of ID2 blocked HIF-1α activation by TGF-β

(Cao, et. al., 2009). In addition, we have previously shown that HIF-1α mRNA is increased in the peritoneum of women with endometriosis and in endometriosis lesions, and this was attributed to increasing TGF-β1 activity (Young, et al., 2014). HIF-1α is routinely degraded in the presence of oxygen through the ubiquitination pathway, whereas hypoxia leads to HIF-1α stabilisation and activity (Semenza, 2012). However HIF-1α has previously been detected in normoxic conditions and although the mechanism for this normoxic stabilisation remains unclear, it is thought that increasing mRNA expression can overcome the protein degradation (Basu, et al., 2011). In the current study we have shown that TGF-β regulates HIF-1α at the transcriptional level through decreased expression of ID2 under normoxic conditions. This observation underpins a potential regulatory pathway for TGF-β induced HIF-1α expression and induction of a Warburg-like effect in endometriosis. Furthermore, studies have shown HIF-1α to regulate expression of ID2, indicating a potential feedback cycle of expression (Lofstedt, et al., 2004).

TGF-β1 has been shown to be upregulated in the peritoneal fluid from women with endometriosis (Oosterlynck, et al., 1994, Young, et al., 2014). A recent study has shown that TGF-β deficiency in the peritoneum of mice reduced lesion development 11-fold (Hull, et al., 2012). Moreover, the role of TGF-β in ovarian function has been established and is emerging as a new therapeutic for manipulating ovarian function and improving fertility (Knight and Glister, 2006). Thus, the TGF-β pathway may be a potential fertility-sparing therapeutic target for endometriosis.

In conclusion, we present preliminary evidence that TGF-β1 influences *ID2* expression in peritoneal mesothelial cells. We also show here that ID2 influences HIF-1α expression in peritoneal mesothelial cells. Taken together with our previous findings showing that an increase in HIF-1α increases local lactate production, these results suggest that TGF-β1 regulates changes in the metabolic phenotype of peritoneal mesothelial cells via HIF-1A and through the ID2 pathway. Increasing lactate production by the peritoneum may facilitate ectopic endometrial cell survival and invasion into the peritoneal mesothelium, promoting the development and progression of endometriosis.

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**Authors' roles**

AWH, WCD and VJY conceived and designed the project. VJY carried out the laboratory work. VJY, SFA and JKB carried out the analysis. All authors contributed to the manuscript write up.

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**Conflict of interest**

The authors have no conflicts of interest.

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449

450 Figure legends

451

452 **Figure 1. Immunohistochemistry of paraffin embedded sections of human**
453 **peritoneum.**

454 Inhibitor of DNA binding protein 2 (ID2) protein expression is localised to the
455 mesothelial cells of peritoneum in women with **(A)** and without **(B)** endometriosis
456 (red arrows)**.** Peritoneum adjacent to endometriosis lesions expressed significantly
457 lower levels of *ID2* mRNA when compared to peritoneum collected distal from
458 endometriosis lesions **(C).** Effect of transforming growth factor (TGF)-β1 treatment
459 on *ID2* gene expression in human peritoneal mesothelial cells (HPMC) treated with
460 2ng/ml TGF-β1 for 12 hours: TGF-β1 treatment significantly decreased *ID2* mRNA
461 expression (**$p<0.01$ versus control, $n=6$) **(D).** Effect of TGF-β1 treatment on *ID2*
462 gene expression in immortalized mesothelial (MeT-5A) cells treated with 2ng/ml
463 TGF-β1 at different time points **(E).** TGF-β1 treatment significantly decreased *ID2*
464 mRNA expression in the MeT-5A cells at 6, 12 and 24 hours after treatment when
465 compared to no treatment control cells at the same time point (data in D & E are
466 mean+/- *SEM, **$p<0.01$,***$p<0.001$, $n=3$*). Scale bar: 20μm

467

468

469 **Figure 2. siRNA knockdown of ID2 in MeT-5A cells.**

470 MeT-5A cells were treated with siRNA for 48 hrs for gene expression studies. **(A)** A
471 representative western blot and summary graph *(n=3)* of siRNA knockdown of ID2,
472 or scrambled siRNA control, and effect of TGF-β1 on ID2 protein expression. **(B)**
473 siRNA knockdown of ID2 resulted in a significant decrease in *ID2* expression when

compared to scrambled siRNA in the MeT-5A cells. *(*data are mean +/-SEM, **p<0.05, n=3).*

**Figure 3. Effect of ID2 knockdown on HIF-1α and lactate secretion in MeT-5A cells**.

**(A)** ID2 siRNA significantly increased hypoxia inducible factor- 1α (HIF-1α) mRNA expression in MeT-5A cells when compared to MeT-5A cells treated with scrambled siRNA *(*mean +/-SEM, **p<0.05, n=3*). **(B)** ID2 siRNA significantly increased lactate concentrations in cell culture media from MeT-5A cells when compared to cell culture media from MeT-5A cells treated with scrambled siRNA *(*mean +/- SEM, **p<0.05, n=3*). TGF- β 1 showed a non-significant increase in HIF-1 α mRNA expression and lactate secretion, alone or in combination with ID2 siRNA.

**Supplementary Figure 1: Effect of anti-TGF-β1 treatment on MeT-5A cells exposed to TGF-β1.**

Cells were pre-incubated with anti-TGF-β1 followed by treatment with TGF-β1 (2ng/ml) for 60 minutes and immunostained for pSmad 2/3. Cells treated with TGF-β1 only showed a noticeable increase in pSmad 2/3 expression and protein was seen to relocate to the nucleus of cells when compared to cells blocked with anti-TGF-β1 (n=3). Scale Bar = 100μm

Table 1. siRNA oglinucleotide sequences used in a study of the roles of ID2 and transforming growth factor-β1 in endometriosis in women.

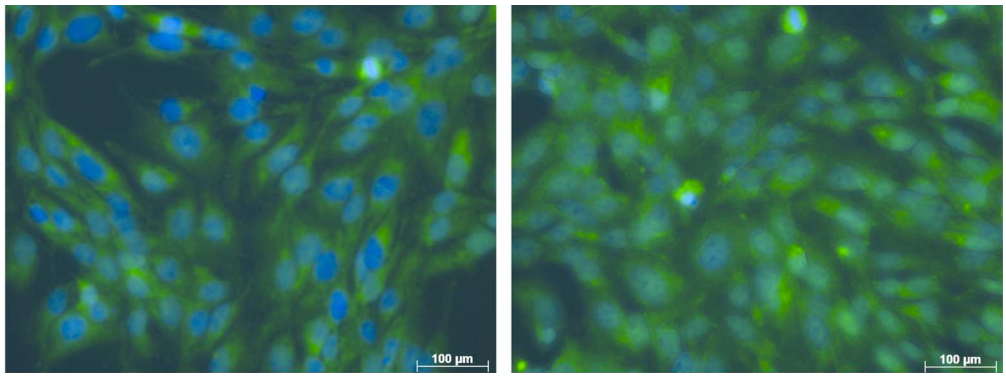
| siRNA | Direction | Sequence 5' – 3' |
|---|---|---|
| ID2 | Sense | GAAUUCCCUUCUGAGUUAATT |
| | Anti-sense | UUAACUCAGAAGGGAAUUCAG |
| ID2 | Sense | CGAUGAGCCUGCUAUACAATT |
| | Anti-sense | UUGUAUAGCAGGCUCAUCGGG |

ID2: inhibitor of DNA binding protein 2

Table 2. Primers used for quantitative RT-PCR. All primers were pre-validated and supplied by Primer Design.

| Gene of interest | Direction | Primer sequence 5' to 3' |
|---|---|---|
| *ID2* | Sense | CGATGAGCCTGCTATACAACA |
| | Anti-sense | AGGTCCAAGATGTAGTCGATGA |
| *HIF-1A* | Sense | TGCCACATCATCACCATATAGAG |
| | Anti-sense | TGACTCAAAGCGACAGATAACA |

HIF-1α: hypoxia inducible factor α




| | | |
|---|---|---|
| Anti TGF-β1 | + | – |
| TGF-β1 | + | + |

74x34mm (600 x 600 DPI)

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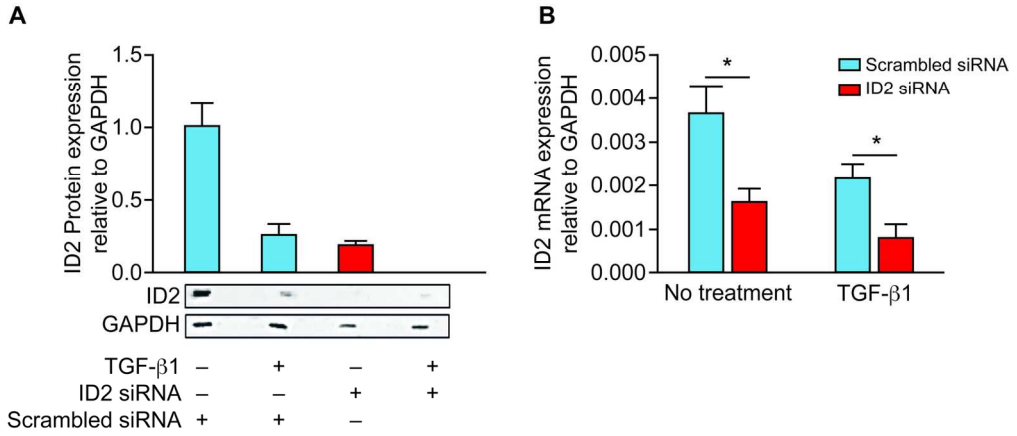


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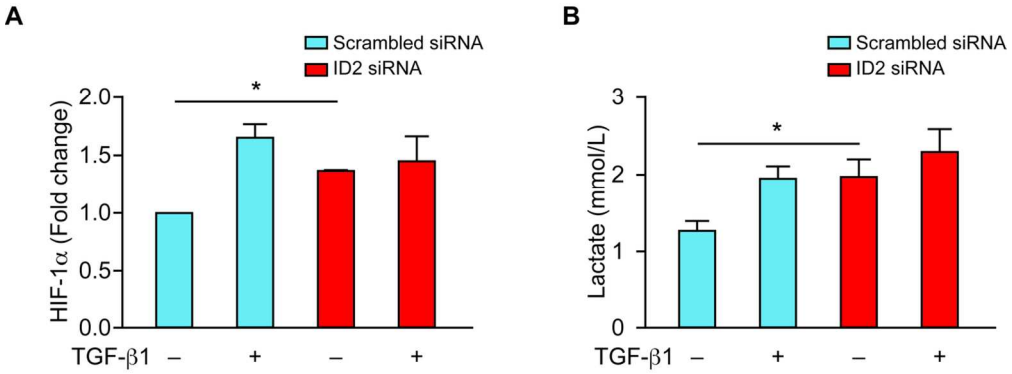


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